Microbial source tracking

Update on genetic markers project

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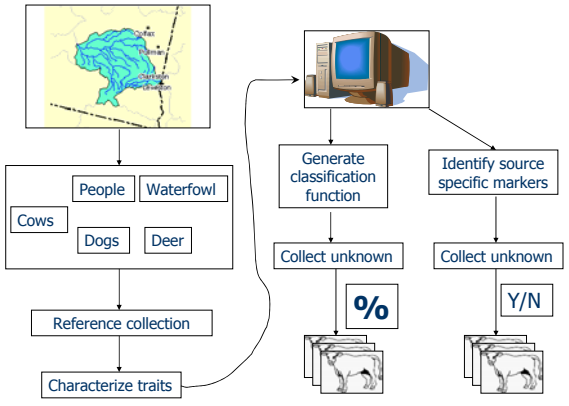
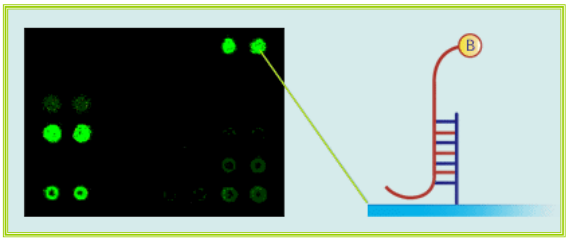
Goal:

Identify genetic markers that are suitable for apportioning fecal pollution to original sources.

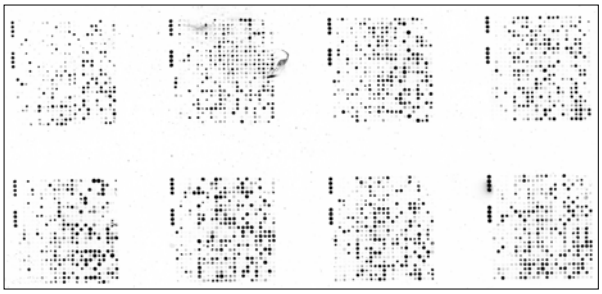


What is a microarray?



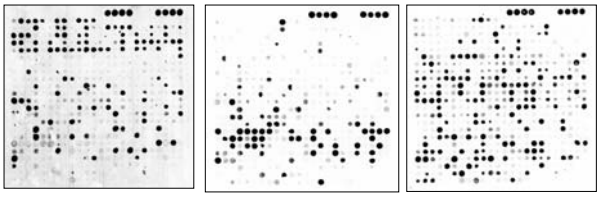
Probes are “printed” on the slide
Targets are detected after hybridization

Example hybridization with mixed-genome, *Enterococcus* microarray



(n = 4,320 probes)

Human isolate | Elk/deer isolate | Cow isolate



- Collected data from 390 hybridizations
- 4,320 markers screened per hybridization

PCR Validation		Pooled		
Marker	Host	Original	USGS	Enrichments
15	Cow	√	√	√
19	Cow	√	√	√
18	Cow	√	√	√
77	Human	√	√	√
68	Human	√	---	---
81	Human	√	---	---
66	Human	√	---	---
67	Human	√	---	---
37	Cervid	√	---	---
40	Cervid	√	---	---
31	Cervid	√	---	---
48	Cervid	√	---	---
None	Dog			
None	Waterfowl			

PCR validation summary

- 12 markers validated at local level; 4 at national level
- Markers may not be present in every individual
- Many of the markers are related to carbohydrate metabolism

What is next?

- Develop a quantitative assay using the 12 genetic markers
- Examine source, fate, and transport of *Enterococcus* markers in surface waters
- Use assay to apportion fecal pollution amongst sources

Collect water samples

Isolate *Enterococcus* on selective media

Extract DNA from isolates

Genotype 96 isolates per sample

Provides ability to detect strains given *ca.* 5% prevalence in sample

Competitive grant renewal

1. Test assumptions about BST:
   - Host specificity exists --- √
   - Shedding constant within & between individuals
   - No significant environmental replication
   - No significant difference in survivorship
2. Quantify real-world samples (Colville?)

Acknowledgements

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•Frank Loge, Civil & Environ. Eng., Pullman
•John Gay, VCS & FDIU, Pullman

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•Tom Besser, VMP & WADDL, Pullman
•Monica Borucki, USDA-ARS, Pullman

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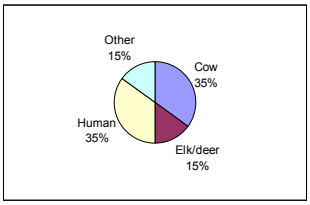
Staff & others:
- Marilyn Soule, Ph.D.
- Melissa Krug
- Stacey LaFrentz
- Melissa Oatley
- Field Disease Investigation Unit

Needed: fecal samples from around the country. Please contact: Doug Call, drcall@wsu.edu, 509-335-6313

	Cow	Elk	Human	Other
Cow	80%	1%	2%	17%
Elk	6%	92%	0%	2%
Human	4%	4%	61%	31%
Other	15%	1%	3%	81%

Overall % correct classification = 72.6%



Confidence intervals?